THE GROWTH OF CITRUS SEEDLINGS AS INFLUENCED BY ENVIRONMENTAL FACTORS[1]

BY

RAYMOND E. GIRTON

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Environment plays a large part in the life of plants as well as of human beings. Of the many factors of environment, those selected for the present investigation were temperature, hydrogen-ion concentrations, oxygen, and mixtures of gases.

EXPERIMENTAL TECHNIQUE

The experiments were conducted under continuous artificial illumination and an effort was made to maintain uniform temperatures and humidity. The first series of experiments was conducted in a saturated atmosphere, but all subsequent experiments were carried out under a relative humidity of approximately 50 to 60 per cent. In addition, control cultures were maintained whenever possible in order to provide an adequate basis for comparison.

The periods over which these experiments were continued were not long, since two weeks usually sufficed to bring out distinct differences between the root growth of the treated plants and that of the controls.

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Ordinarily this period also allowed time, under favorable growth conditions, for the production of a second pair of leaves. All roots were coated lightly with a suspension of carbon black when the experiments were set up. This made it possible to distinguish all subsequent growth by its natural whitish color as contrasted with the darker coated regions.

When solution cultures were required, a form of Hoagland's nutrient solution (pH 5.2) was used. This solution was modified by the use of a half-and-half mixture of mono- and dibasic potassium phosphates instead of the monobasic form alone as ordinarily employed. Thus a less acid reaction was obtained (pH 6.0) which was more favorable to the growth of citrus roots. The solutions were not changed during the experiments, but iron was added two or three times each week in the form of a dilute solution of ferric tartrate.

The production of root hairs was measured by means of an index expressing their relative abundance. This index, called the 'root-hair index,' indicated the average number of root hairs per centimeter along one side of a rootlet. This index was obtained in the following manner. Estimates were made of the relative abundance of root hairs by classifying each plant examined on the basis of the following classes: I = very few root hairs, II = few, III = moderate number, IV = abundant, and V = very abundant. It was found by actual count that the average root-hair indexes for these classes had the following approximate values (see table 1): I = 5, II = 15, III = 30, IV = 60, and V = 120.

TABLE 1

APPROXIMATE r. h. i. (ROOT-HAIR INDEX) RELATIONSHIPS

Class	Values of r.h.i. determined by actual count		Approximate r.h.i. (assigned values)
	Number of plants examined	Index	
I	20	4.5 ± 0.4	5
II	43	14.5 ± 0.5	15
III	40	33.9 ± 1.4	30
IV	16	59.8 ± 4.4	60
V	2	119	120

The approximate root-hair index for a given population of plants could therefore be determined by: first, a microscopic examination in which each plant was classified according to the estimated abundance of root hairs; second, the number of plants within each class was

multiplied by the assigned index value for the class; and third, the resulting products were added and the sum divided by the total number of plants. This gave a quantitative index for the relative abundance of root hairs which was based upon estimates of the individual plants. The following formula expresses in a condensed form the relationships just discussed.

$$I_A = \frac{\Sigma\ (a_I \,.\, n_I + a_{II} \,.\, n_{II} \ldots \ldots + a_V \,.\, n_V)}{N}$$

I_A = approximate root-hair index.

a_I, a_{II}, etc. = average root-hair indexes for the respective groups I, II, etc.

n_I, n_{II}, etc. = number of plants falling within the designated group.

N = total number of plants producing root elongation under the experimental conditions.

A simple example may serve to illustrate the method. Suppose that in a population of 30 plants, it was determined by a microscopic examination of the roots that the plants should be classified as follows: 3 in class I, 7 in class II, 15 in class III, and 5 in class IV. Then the approximate values for the different classes would be: 3 (the number of individuals in the class) × 5 (the approximate *r.h.i.* for the class) = 15 for class I, 7 × 15 = 105 for class II, 15 × 30 = 450 for class III, and 5 × 60 = 300 for class IV. The sum of these products (870) divided by the total number of plants (30) gives the approximate root-hair index for the entire population (29). Substitution in the suggested formula would give the same result.

$$I_A = \frac{\Sigma\ (3.5 + 7.15 + 15.30 + 5.60)}{30} = 29$$

ENVIRONMENTAL FACTORS

Temperature

Seedlings of the grapefruit (*Citrus maxima* (Burm.) Merrill), the sour orange (*Citrus aurantium* L.), and the sweet orange (*Citrus sinensis* Osbeck) were grown at constant temperatures in a differential thermostat placed at the writer's disposal by the Division of Plant Pathology of the Citrus Experiment Station. This apparatus is similar to the one described by Livingston and Fawcett (1920). It contains seven cylindrical compartments, 40 centimeters in diameter and 45 centimeters in depth, with maintained temperatures graded from about 12° to 35° C, depending upon the adjustment and the

manner of illumination. Each compartment was covered with a lid made of two thicknesses of window glass with a 3-centimeter air space intervening. An electric light suspended above each compartment furnished continuous illumination. In the first trial, with grapefruit plants, 75-watt lamps were employed, but these were too weak for the best photosynthetic activity and were replaced by 250-watt lamps in the subsequent experiments.

GRAPEFRUIT SEEDLINGS

Four- to five-weeks-old seedlings were selected for comparative uniformity of top and root development. They were supported in paraffined cork stoppers and placed in one-quart jars, each culture jar containing six seedlings. Five jars, containing a total of 30 plants, were then placed in the apparatus and maintained at the following temperatures: 11°, 14½°, 19°, 22½°, 26°, 30°, and 34° C, respectively. Continuous illumination was supplied by 75-watt 'daylight' electric lamps suspended about one meter above the plants, one lamp to each compartment. The plants were removed at the end of the third week and examined for increase in root length and production of root hairs (table 2). The examination showed that the root growth, which was confined to elongation of the tap roots, and the root-hair production were greatest at 26° C and consistently decreased with higher or lower temperatures. The course of the growth is shown by the graphs in figures 1 and 2.

TABLE 2

ROOT GROWTH OF GRAPEFRUIT SEEDLINGS AT DIFFERENT TEMPERATURES FOR A THREE-WEEKS' PERIOD

Usual temperature	Temperature fluctuations*	Number of plants	Entire root elongation	Root elongation per plant	Root-hair index
° C	° C		cm.	cm.	
11	10 -16½	30	2 9	0 10	7
14½	13½-19	30	10.8	0.36	8
19	18 -22	30	10 8	0 36	24
22½	21½-25½	30	19 2	0 64	47
26	25 -28½	30	30 2	0 97	78
30	28½-31½	30	23.7	0 79	58
34	32½-35½	27	9 2	0.34	8

* Temperature fluctuations recorded in this column were due in part to the stoppage of the cooling apparatus for a short time.

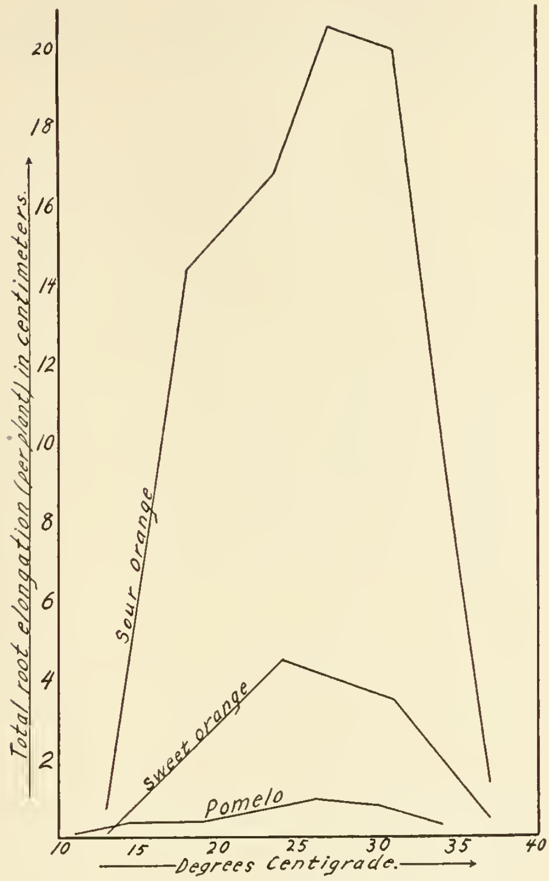


Fig. 1. Graphs showing average root growth of citrus seedlings grown at various maintained temperatures.

SOUR-ORANGE SEEDLINGS

It may be seen from table 3 and from figures 1 and 2, that the total root elongation of the sour-orange plants followed the trend previously noted for the grapefruit seedlings, i.e., increased growth with increased temperature up to a point of maximum growth and a rapid decrease in growth with a further increase in temperature. The elongations of the tap and lateral roots were roughly parallel over the entire temperature range. On the other hand, the number of lateral roots produced showed no relation to the temperature. The considerably greater root elongation obtained with sour orange as compared with that obtained with grapefruit was due largely to the increased illumination in the sour-orange experiment.

The production of root hairs exhibited a temperature relation similar to that of root elongation. The optimum temperature, however, was somewhat higher.

TABLE 3

GROWTH OF SOUR-ORANGE SEEDLINGS AT DIFFERENT TEMPERATURES FOR A TWO-WEEKS' PERIOD

Temperature		Number of plants	Stem		Leaves		Tap root elongation	Lateral roots		Total root elongation	Approximate root-hair index
Usual	Fluctuations*		Diameter	Height	Number	Fresh weight		Number	Elongation		
° C	° C		cm.	cm.		gm.	cm.		cm.	cm.	
13	12½–16½	30	18± .002	7.3±0.11	2 1±0 01	.12± 004	0.3±0 03	2 6±0.36	0 5±0.07	0 8±0 10	1±0.2
18	17½–20½	30	18± .003	7 8±0.12	2 1±0 01	.13± 003	1 9±0 14	11 1±0.67	12 4±1.12	14 3±1 17	6±0.4
23½	23 –25	30	.18± 002	7.9±0.13	3 0±0 10	.13± 005	3.8±0 22	7 0±0.53	13 0±1 28	16.8±1 32	13±1.4
27	26½–28½	30	.17± .002	7.9±0.11	3 5±0 08	17± 008	6 3±0 33	7 1±0 63	14.2±1 39	20 5±1 43	21±2 3
31	30½–32½	30	.16± .002	8 2±0.15	3 5±0.08	.19± 009	6.6±0.31	7 1±0 50	13.3±1.08	19 9±1 06	25±1.6
34	33½–35	30	.17± .002	7 6±0 12	3.0±0.06	14± 005	5.2±0 37	3 0±0 46	5 0±0 86	10 2±1.04	35±3.0
37	36–38	30	.16± .002	7.3±0 14	2.3±0.04	11± 003	0.4±0 04	5.9±0.76	1 0±0.14	1.4±0.15	1±0.3

* The larger fluctuations with the lower temperatures were due to the stoppage of the cooling apparatus for a short time.

SWEET-ORANGE SEEDLINGS

This experiment was set up with one-month-old sweet-orange plants in a manner similar to that described for the preceding experiments. In this case, however, all cotyledons were removed for the purpose of equalizing the individual supplies of stored food. Owing to a shortage of suitable plant material only four sets of 30 plants each were employed. The temperatures were 13°, 24°, 31°, and 37° C. After two weeks the plants were examined and measurements of the growth were made (table 4).

TABLE 4

GROWTH OF SWEET-ORANGE SEEDLINGS AT DIFFERENT TEMPERATURES FOR A TWO-WEEKS' PERIOD

Temperature	Number of plants	Stem		Leaves		Tap root elongation	Lateral roots		Total root elongation	Root hairs	
		Diameter	Height	Number	Fresh weight		Number	Elongation		Number of plants*	Approx. index
° C		cm.	cm.		gm.	cm.		cm.	cm.		
13	30	.16± 002	6.5±0 12	2.1±0.02	08±.005	0 1±0.01	0 0	0.0	0 1±0.01	11	7±3
24	30	.16±.003	6.7±0 18	2.7±0.09	09±.005	2 8±0.21	1.3±0 28	1 6±0.39	4 4±0.49	29	25±3
31	30	.15±.002	6.6±0.18	2.9±0 06	.09±.006	3 3±0 34	0 1±.005	0 1±0 03	3 4±0.36	24	60±5
37	30	.15±.002	6.4±0 17	2.4±0 06	06± 004	0.4±0 06	0 1±0 03	0 1±0.01	0.5±0.06	20	8±2

* Many of the plants at the extreme temperatures produced no root elongation during the experiment and were thus automatically eliminated from the root-hair study.

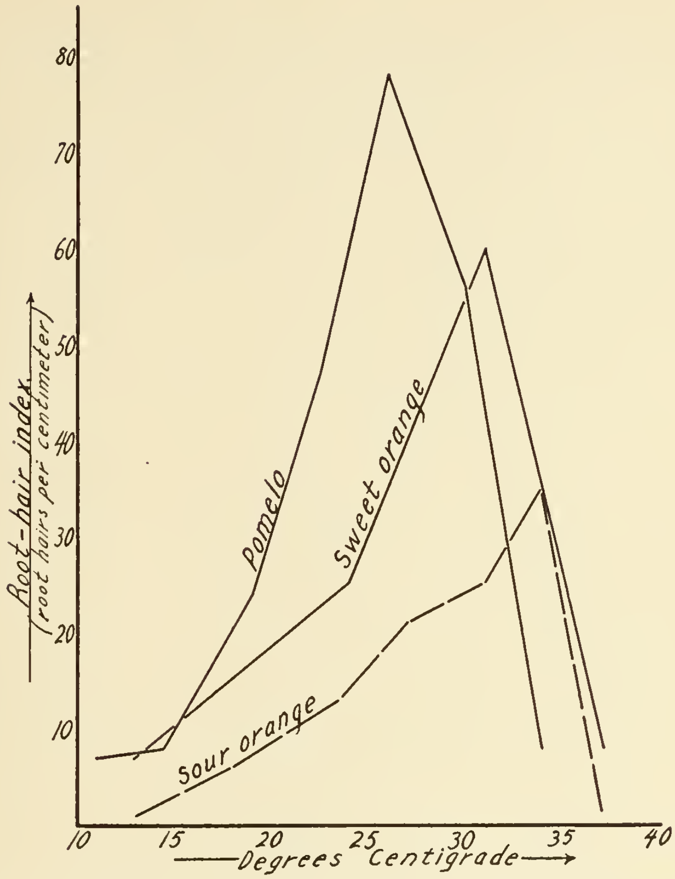


Fig. 2. Graphs showing root-hair production with citrus seedlings grown at various maintained temperatures.

The curves for the sweet-orange plants (figs. 1 and 2) follow a course similar to that described for the grapefruit and the sour-orange plants. The optimum temperature for root-hair production appears to be somewhat higher than that for total root elongation. A study of the tabulated data for this experiment furthermore suggests that the top development, as evidenced by the number and fresh weight of the leaves produced, was influenced to a limited extent by the temperature, even though the experimental period was short.

DISCUSSION

The preceding experiments indicate that there exists a minimum, an optimum, and a maximum temperature for root elongation and the production of root hairs. The minimum temperature under the experi-

mental conditions was roughly 12°, the optimum 26°, and the maximum 37° C. The fact that the apparent optimum for the sweet-orange plants was about two degrees lower than the average value stated, is probably due to the greater temperature intervals used in that experiment. Also, the somewhat lower maximum temperature apparent for the grapefruit seedlings may be explained upon the basis of insufficient illumination, resulting in a low photosynthetic activity which was here coupled with a high respiratory rate. The average temperature values are in accord with those found by Peltier (1920) for the top growth of grapefruit plants. Using five-degree temperature intervals Peltier found 15° C for the minimum, 20 to 30° for the optimum, and 35° C for the maximum temperatures.

It is of interest to note that the optimum temperature did not lie halfway between the minimum and maximum temperatures, but rather closer to the maximum end of the range. This is especially noticeable in the case of the temperatures found most favorable for root-hair production, the apparent optima in this case being slightly higher than those obtained for root elongation.

Although the periods used for experimentation were short, some responses were obtained with the slower growing tops. The number and fresh weight of the leaves showed a behavior similar to that of the roots in relation to temperature. The plants maintained at moderate temperatures were able to start the development of a second pair of leaves, but those exposed to the extreme temperatures were unable to do so.

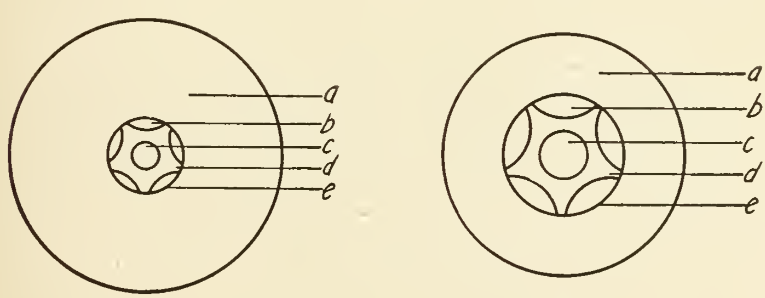
Finally it may be observed that the temperature coefficient is of considerable magnitude for the different phases of root growth at the lower temperatures, but decreases rapidly with increased temperatures and approaches zero at the highest temperatures (Fawcett, 1921).

HYDROGEN-ION CONCENTRATIONS

The influence of the reaction of the culture solution on root growth was studied in several series of cultures of sour-orange seedlings. The original reaction of the nutrient solution (pH 6.0) was changed by adding hydrochloric acid or sodium hydroxide. The colorimetric method was employed for the determination of the pH value of all solutions.

SOLUTIONS AT pH 4.0 TO 9.0 WITH DAILY ADJUSTMENTS OF THE pH (PRELIMINARY EXPERIMENT)

Four sour-orange plants were grown in each of six 2-quart jars which contained solutions adjusted to the following pH values: 4, 5, 6, 7, 8, and 9. Daily pH determinations were made with all solutions and readjustments were made where necessary. It was observed that the plants acted upon the solutions in such a way as to change the pH toward 6. The daily change in the acid solution was slight, but that in the neutral and the alkaline solutions was considerable, amounting in one case to 0.9 pH. Undoubtedly the excretion of carbon dioxide by the plant roots was largely responsible for the changes observed. After three weeks' growth, a peculiar thickening was noticed in the ends of many of the roots growing in the solution at pH 4. Sections were made of these abnormal portions and it was found that the increased size was due to a considerable increase in the cortical tissue. A cross-section taken through the thickened region of one of these roots is represented by the drawing in figure 3. It may be observed that the central tissues of the root, particularly the xylem and the phloem, were still in the primary state characteristic of a very young and healthy root. The cortical layer, however, was much thicker than that of a normal root and occupied the greater portion of the cross-sectional area. A cross-section of a normal root of the same species is represented for comparison in figure 4.



A B

Fig. 3. (A) Diagram of a cross-section of a sour-orange root showing relative development of cortex and stele from a culture solution having a reaction of pH 4.0.

(B) Diagram of a cross-section of a normal sour-orange root. a = cortex; b = phloem; c = pith; d = xylem; e = pericycle.

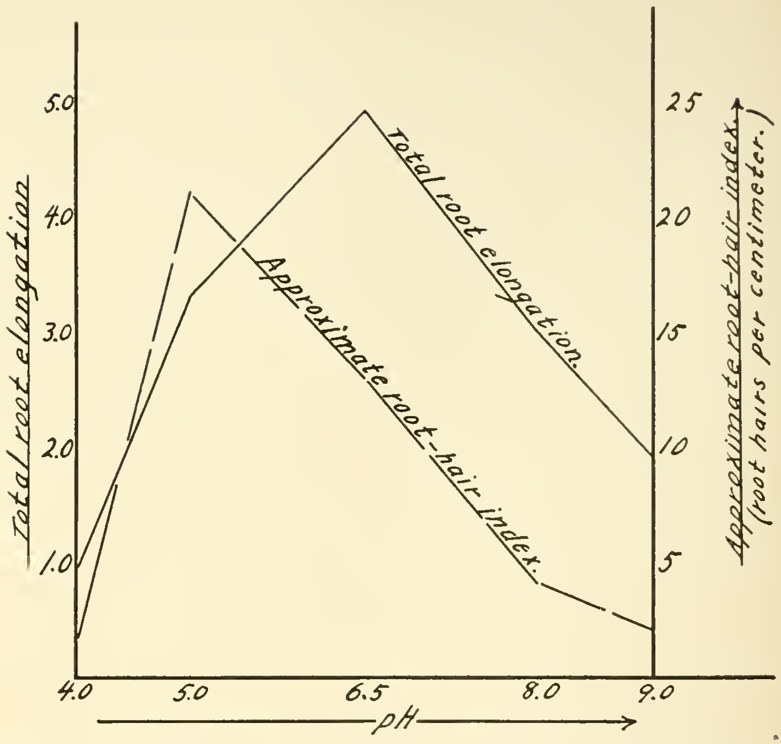


Fig. 4. Graphs showing root growth and root-hair production with sour-orange seedlings grown in solution at various hydrogen-ion concentrations.

GROWTH IN SOLUTIONS AT pH 5.0 TO 9.0 WITH DAILY ADJUSTMENTS OF THE pH

An experiment with six-weeks-old plants was set up similarly to the one just described, using five cultures of 30 plants each, in glass jars holding three and one-half liters of solution. The solutions were adjusted to the values: pH 5.0, 6.0, 7.0, 8.0, and 9.0. It was observed that light colored precipitates were formed in the solutions of pH 8.0 and 9.0. These precipitates were probably largely composed of calcium and iron phosphates, as these substances are relatively insoluble in alkaline solutions. The cultures were maintained at 29 ± 1° C and received continuous illumination from a 500-watt light suspended about one-half meter above the plants.

An examination of the plants after a growth period of two weeks brought out the following facts. In general, the development of the root systems was fairly uniform over the entire pH range. The most

abundant production of root hairs, however, occurred at pH 5.0 and 6.0, viz., 33 ± 3 and 28 ± 3, respectively. The smallest number (11 ± 3) was produced in the most alkaline solution (pH 9.0). Owing to the occurrence of large daily variations (0.5 to 0.8 pH) in the alkaline solutions, it is evident that much of the growth was made in solutions closer to neutrality than the initial pH values indicate.

SOLUTIONS AT pH 4.0 TO 9.0 WITH pH ADJUSTMENTS MADE TWICE DAILY

This experiment consisted of a repetition of the preceding one with a few modifications. The extent of the pH range was increased to include a more acid solution, i.e., pH 4.0. Because of the rapidity with which the alkaline solutions underwent change, it seemed desirable to adjust the hydrogen-ion concentrations twice each day. These adjustments were made at 9 A.M. and 5 P.M.

The plant material employed in this experiment consisted of seven-weeks' old sour-orange seedlings. These plants were carefully selected in five lots each in order to obtain comparable populations for the different treatments. Continuous illumination from a 500-watt light was again used but the temperature was reduced to $25 \pm 1°$ C. The experiment extended over a period of 10 days.

The data in table 5 show that the maximum growth of roots occurred in solutions having reactions of pH 6.5. The production of root hairs also shows a correlation with the hydrogen-ion concentration. From the graph in figure 5 it may be seen that the production of root hairs was strongly depressed at pH 4.0, rose to a maximum at pH 5.0, and declined steadily with the higher pH values. The optimum hydrogen-ion concentration for the production of root hairs was considerably greater than that for root elongation, the latter being situated near the point of neutrality.

DISCUSSION

The condition permitting maximum root growth was found to exist in the region of neutrality. The most favorable range for root-hair production, however, occurred in a distinctly acid solution (pH 5.0). Both the production of root hairs and the total root elongation showed evidence of depression in the most acid (pH 4.0) and the most alkaline solutions (pH 9.0).

TABLE 5

GROWTH OF SOUR-ORANGE SEEDLINGS IN SOLUTIONS OF DIFFERENT HYDROGEN-ION CONCENTRATIONS

Initial pH	Variations in pH		Number of plants	Stem		Leaves		Tap root elongation	Lateral roots		Total root elongation	Root hairs	
	A.M.	P.M.		Diameter	Height	Number	Fresh weight		Number	Elonga-tion		Number of plants concerned	Approxi-mate index
				cm.	cm.		gm.	cm.		cm.	cm.		
4 0	0 —0 3	0 —0 1	30	.16±.002	7.9±0.09	2 1±0 02	11±.004	0 4±0 06	1 8±0 30	0 5±0 06	0.9±0.14	22	1±0.3
5 0	0 —0 3	0.0	30	17±.002	8 0±0 12	2 4±0.05	12±.005	1 3±0 16	2 6±0.38	2 0±0.34	3.3±0 43	24	21±2 4
6 5	0 0	0 —0.1	30	.17±.001	7.9±0.10	2.2±0 03	.11±.004	1 3±0 13	4 1±0 50	3.6±0.52	4.9±0 59	25	13±1 2
8 0	0 —0 5	0 1—0 5	30	.17± 002	8 0±0.12	2 2±0.03	.11± 004	1 2±0 12	2 2±0 33	1.8±0.33	3.0±0 37	26	4±0.8
9 0	0 1—0.5	0 1—0 5	30	16±.002	7.9±0.11	2 1±0.01	.11±.005	0.9±0.12	1.5±0.25	0.9±0.17	1.8±0 24	27	2±0.4

The fact that comparatively few hairs were produced in the alkaline solutions (pH 8.0 and 9.0) of the preceding experiment is worthy of note in another connection. It is a common belief that Citrus plants do not produce root hairs. At the same time it may be noted that the soils common to the Citrus regions of southern California have a decided alkaline reaction. Therefore it seems that the belief in the non-production of root hairs by Citrus roots may be related to the characteristic alkaline reaction of the soil solution which acts to suppress their formation. This explanation appears to be a logical one and worthy of further experimental study.

OXYGEN

Three similar lots of 30 plants each were placed in 2-quart jars fitted with cork stoppers, each jar containing 10 plants. Three of the jars were filled with nutrient solution which had been previously aerated for three hours. These solutions were also continuously aerated during the period of the experiment. A second lot of plants grew in an unaerated solution, which contained initially a considerable amount of dissolved oxygen. The third lot of plants was placed in jars containing a solution which had been boiled in a partial vacuum to drive out the dissolved air. All jars were sealed with a paraffin-petrolatum mixture (80% paraffin, 20% petrolatum), but the jars of the aerated and control cultures were provided with vents so that the outside air had access to the solutions. The jars containing the evacuated solution were equipped with soda-lime tubes for the purpose of preventing the accumulation of carbon dioxide in high concentrations.

Continuous aeration was secured by the use of a water pump described by Allison (1922). The pump was connected with three glass tubes, each extending into the solution of one of the aerated cultures. Each tube was 26 centimeters long and was provided with a small perforated bulb at the lower end. This allowed the air to be forced down into the solution to a depth of about 20 centimeters and expelled in the form of small bubbles.

Analyses for dissolved oxygen were made of the culture solutions at the beginning and end of each experiment. The method used was that devised by Winkler and described by Treadwell and Hall (1915). The technique given by Allison and Shive (1923*a*) for applying this method to small quantities of solution was followed. Some disparity was observed in the actual amounts of dissolved oxygen found in the different experiments. This lack of agreement may have been due to

the temperature differences and to possible inaccuracies in the N/25 sodium thiosulfate solution required for titrating the iodine liberated by oxidation. In order to correct this situation the analyses in the third experiment were conducted at the temperature at which growth took place, and a freshly prepared N/25 thiosulfate solution was used. It is felt that this latter set of analyses is reasonably trustworthy and representative.

AERATION CONDUCTED AT 27 ± 1.5° C

Two-months-old plants were employed in an experiment extending over a period of 12 days. Illumination was supplied continuously by a 500-watt light suspended about one-half meter above the plants. On the sixth day the soda-lime associated with the boiled-solution cultures was removed. The experimental results are described briefly in the following paragraphs.

The analyses of the original solutions showed that the preliminary aeration of the culture solution had little effect in increasing its oxygen content. Apparently the pouring and shaking attendant to the preparation resulted in the incorporation of nearly sufficient oxygen to saturate the solution. The treatment of combined boiling and evacuation, however, decreased the oxygen content considerably, although there was still a significant amount of dissolved oxygen left in the solution.

The results of the analyses at the end of the experimental period reveal two interesting points. First, the oxygen content of the boiled solution remained practically constant. It is certain that the outer air had access to these solutions in spite of the seals used, and that withdrawal of oxygen from the solutions by the roots was coupled with an equally rapid absorption of oxygen by the solutions from the air. Second, the oxygen content of the control culture solutions had been eventually reduced to that of the boiled solutions, apparently by the action of the plant roots.

No significant differences in plant growth were evident between the control and the boiled-solution cultures. Since the original differences in oxygen content were not maintained, it is probable that the amounts of dissolved oxygen in the two series of solutions were very similar over the major part of the experiment. An unfortunate fungus infestation retarded the growth of the aerated plants. For this reason all plants receiving aeration had to be discarded at the end of the experiment.

TABLE 6

THE EFFECT OF AERATION UPON THE GROWTH OF SOUR-ORANGE SEEDLINGS

Treatment	Oxygen per liter		Number of plants	Stems		Leaves		Tap root elongation	Lateral roots		Total root elongation	Root-hair index
	At beginning	At end*		Diameter	Height	Number	Fresh weight		Number	Elonga-tion		
	cc.	cc.		cm.	cm.		gm.	cm.		cm.	cm.	
Aerated	5.59 5.35 } 5.47	6.34 6.34 } 6.52 6.89	20†	.17±.003	11.5±0.19	4.6±0.07	.31±.019	5.4±0.72	7.8±0.34	17.4±1.03	22.8±0.97	27±2.8
Control	5.23 5.18 } 5.20	4.85 5.04 } 4.86 4.70	30	.17±.002	11.2±0.14	4.5±0.07	.32±.013	2.2±0.25	8.3±0.46	15.1±0.94	17.3±0.90	4±0.6
Boiled	3.48 3.64 } 3.56	4.61 4.70 } 4.81 5.11	29	.17±.001	11.4±0.12	4.5±0.10	.30±.013	1.9±0.27	9.1±0.33	17.5±0.79	19.4±0.85	2±0.3

* Analyses of solutions in individual jars, three jars in each treated series.

† The plants in one of the aerated jars were discarded because of fungus infestation.

AERATION CONDUCTED AT 25 ± 1° C

In an experiment using the methods just described, two-months-old sour-orange plants were grown for about three weeks (table 6).

In the control solutions the original oxygen content was very close to that of the solutions aerated for three hours, as noted in the previous experiment, showing that a high degree of aeration was secured during the course of preparation. During the course of the experiment the action of the roots upon the control solution eventually reduced its oxygen content to that of the boiled solution, which had been increased by the absorption of atmospheric oxygen.

Colorimetric determinations were made of all solutions at the close of the experiment in order to determine the hydrogen-ion concentrations. Those of the aerated solutions were pH 6.4, 6.5, and 6.6, those of the control solutions 6.3, 6.4, 6.3, and those of the boiled solutions 6.3, 6.3, 6.3. The slightly higher values for the aerated cultures suggest that aeration had removed some dissolved carbon dioxide from these solutions.

Table 6 shows that the plants of the control and boiled-solution cultures were characterized by a very similar development. As no differences greater than three times the probable error are evident, it may be concluded that no significant differences existed. In the case of the aerated cultures, however, the increase of tap-root elongation may be of significance; in fact the longer tap-root development attained by the aerated cultures was obvious when the plants were examined.

The greater relative number of root hairs produced in the aerated cultures is very evident from the data presented in this table. It seems that root-hair production is much more sensitive to aeration than is the elongation of the tap or lateral roots.

Table 7 gives the results of experiments in which the boiled-solution cultures were omitted. The presence of fungi upon the roots of plants in the aerated cultures suggested to the writer that fungus spores were being carried into the aerated solutions by way of the air stream. In order to remedy this trouble a loose cotton filter and a wash bottle containing distilled water were inserted in the air line between the pump and the aerated cultures. This device proved to be effective, as all aerated-culture plants subsequently remained entirely free from fungus attack.

TABLE 7

THE EFFECT OF AERATION UPON THE GROWTH OF SOUR-ORANGE SEEDLINGS

Treatment	Oxygen per liter		Number of plants	Stem		Leaves		Tap root elongation	Lateral roots		Total root elongation	Approximate root-hair index
	At beginning	At end*		Diameter	Height	Number	Fresh weight		Number	Elongation		
	cc.	cc.		cm.	cm.		gm.	cm.		cm.	cm.	
Aerated	5.78, 5.76, 5.76 } 5.77	5 43±0.03	30	.16±.002	7.8±0.11	3.2±0 10	.14±.004	4 1±0.32	9 2±0.47	14 4±1 04	18 5±1.23	26±2.3
Control	5 59, 5.71 } 5.65	3.13±0.05	30	.16±.002	7.9±0.11	3.2±0.10	.13±.003	2.4±0.13	9 0±0.50	8.9±0.72	11.3±0 70	6±0.80

* Values given are the average of six analyses.

The data presented in table 7 show that both the tap-root and the lateral-root elongation were increased by aeration of the culture solution. The production of root hairs was greatly increased and appeared to be the most marked result of aeration.

Hydrogen-ion determinations were made of all solutions at the close of the experiment. The values obtained were the following: aerated cultures, pH 6.3, 6.3, and 6.2; control cultures, 6.1, 6.1, and 6.1.

DISCUSSION

In general the preceding experiments have brought out the following facts. In the preparation of the nutrient solution, a certain quantity of oxygen was introduced from the air (5.65 cc. per liter at 25° C—table 7), which was later reduced, presumably by the respiratory action of the plant roots, unless replenished by aeration. This quantity of dissolved oxygen closely approaches that given by Landolt, Börnstein, and Roth (1912) for complete saturation, viz., 5.78 cubic centimeters per liter for a temperature of 25° C.

The development of the plants in the boiled-solution cultures duplicated that of the control cultures within the limits of experimental error. This was true for the growth of all organs observed and suggests that some oxygen was being absorbed from the air by both the boiled and the control solutions. On the other hand, the root growth of the plants in the aerated solutions showed evidence of a greater development than in the unaerated solutions. This was especially noticeable in the case of root-hair production.

In the pH of the aerated solutions there was seen a small but constant trend toward a less acid reaction than in the pH of the control solutions. This suggests the presence of an appreciable amount of dissolved carbon dioxide in the unaerated solutions which resulted in the formation of the bicarbonate ion by its partial dissociation. Therefore, the effect of the aeration treatment appears to be twofold: first, the oxygen supply was constantly renewed; second, the carbon dioxide excreted by the roots was continually driven off by the aerating process. It is very likely that both conditions operated to promote the growth of the plants. Certainly both conditions would favor the respiratory activity of the roots and increased respiration in turn would presumably be associated with increased root activity and growth.

The responses to aeration obtained with the sour-orange plants are in keeping with those observed for many other plants. For example

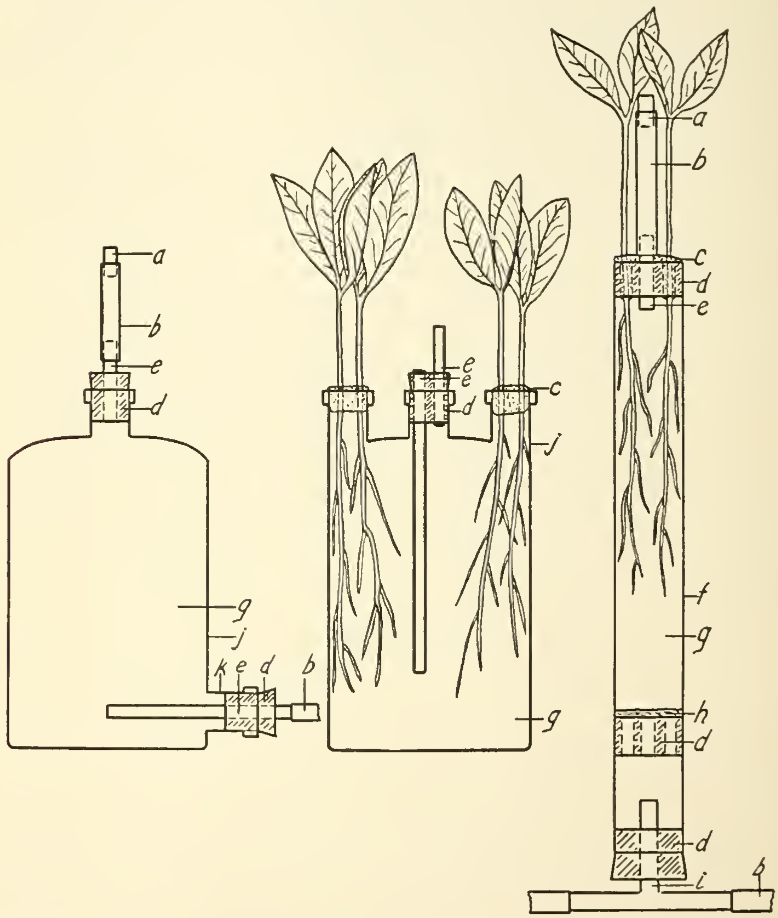
Stiles and Jörgensen (1917) found, with barley and balsam, that an aeration of the culture solutions resulted in increased yields. Andrews and Beals (1919) found that the yield of maize grown in water cultures was also increased by aeration. In addition, the work of Andrews (1920), Allison (1922), Allison and Shive (1923), and Knight (1924) has shown that aeration of solution cultures was beneficial for various plants including oats, peas, soy beans, wallflowers, and mustard. However, not all plants respond to aeration. Free (1917) reported that the growth of buckwheat plants in solution cultures treated with air, oxygen, or nitrogen was equal to that obtained in open controls or in sealed cultures. It is of interest to note that when carbon dioxide was passed through the solutions the plants wilted within a few hours and died within a few days.

A few investigators have also noted the effect of oxygen upon the production of root hairs. Schwarz (1883) observed that a relation existed between the available oxygen supply and the development of root hairs. Miss Snow (1905) found that the root-hair production of corn was suppressed when the roots were exposed to moist air deprived of oxygen, although exposure to moist air was ordinarily accompanied by an abundant production of root hairs. More recently Bergman (1920) found that *Impatiens balsamina* produced root hairs in aerated water.

MIXTURES OF GASES

In order to determine the action of mixtures of gases upon the growth of Citrus roots, a series of experiments was conducted with sour-orange seedlings in cultures of a river sand of medium texture. In the earlier experiments, the plants were grown in opaque triple-necked Woulff bottles. Two small seedlings were placed in each of the outer necks while a rubber stopper fitted with a short glass tube was inserted in the center neck. Half of the jars (usually six) were those having tubulatures at the base (fig. 5). These tubulatures were also fitted with rubber stoppers containing short glass tubes. The jars were connected to a delivery tube of the manifold type, which in turn was connected to a gas-holder and an intervening wash bottle. The gas mixture under investigation was passed through each of the sand-filled Woulff bottles daily by applying a slight suction to the tube extending from the center neck. These openings were kept closed at all other times by means of short pieces of rubber tubing fitted with clamps. Control cultures in a similar set of bottles were maintained in all experiments (fig. 6). These Woulff bottles lacked the

tubulature at the base, and the center neck of each bottle was fitted with a long and a short glass tube instead of only the short one used for the treated cultures. Both of these tubes were open to the air so that a normal diffusion of gases could take place at all times. The



Figs. 5, 6, and 7. Diagrams of apparatus used for experiments on the effect of gases. No. 5, Woulff bottle type. Side view, median section. No. 6, Woulff bottle type as used in control cultures. No. 7, Straight tube type, *a* = glass stopper; *b* = rubber tubing; *c* = wax seal; *d* = rubber stopper; *e* = glass tubing; *f* = glass culture tube; *g* = sand; *h* = glass wool; *i* = T-tube; *j* = Woulff bottle; *k* = tubulature.

sand in all cultures was moistened with nutrient solution at the beginning of each experiment and the plants were customarily sealed in with the paraffin-petrolatum mixture in order to render the system gas tight.

Later on it seemed desirable to employ a container in which a more uniform distribution of gas could be assured, as there was some reason to believe that the composition of the gas drawn off and analyzed from the Woulff bottles was not identical with that surrounding the plant roots. For this reason several of the experiments were conducted with straight-walled glass tubes having an average diameter of about 35 millimeters and a length of 30 centimeters. These cultures

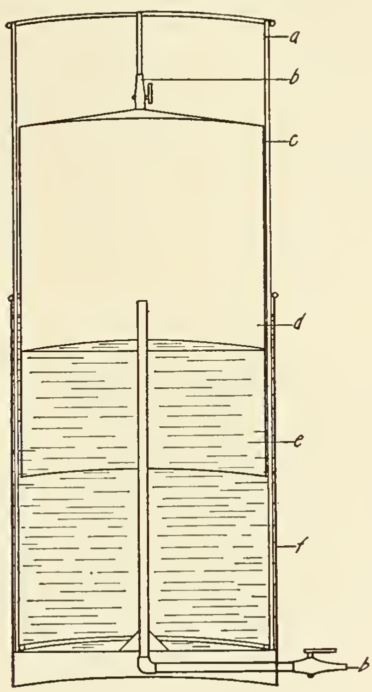


Fig. 8. Gasometer, side view, median section. a = guide for inner tank; b = gas outlet; c = inner tank; d = gas; e = water; f = outer tank.

were set up as shown in figure 7, using two plants to each tube. At the time of planting, the cultures were irrigated with an excess of nutrient solution, the surplus solution being removed by applying a slight suction to the lower outlet tube and drawing several hundred cubic centimeters of air through each tube. The lower outlet tube was then attached to the gas line and the experimental gas mixture drawn through the treated cultures once or twice each day.

The gas-holder (fig. 8) consisted of a galvanized iron tank inverted within a larger tank of the same material. The outer tank was partially filled with water to prevent the escape of gas from the inverted

one. A guide of vertical metal rods was inserted in the space between the two tanks in order to support the inner and movable tank. Gas could be run into or withdrawn from the gasometer by means of a small galvanized iron pipe projecting through the bottom of the outer tank and extending above the water level. There was also a second outlet at the top of the inner tank which was closed by a stopcock.

Analyses were made at intervals (usually every four days) of the gas drawn off from the treated cultures and from the gasometer. All such analyses were made with a portable Haldane apparatus (Haldane, 1920). In addition, at the end of each experiment, a set of analyses was made of the gas from the control cultures. Plant measurements including both root and top growth were also taken at this time. Owing to the presence of soil incrustations on the surface of the roots and to the likelihood of breakage during removal from the soil, no attempt was made to study the production of root hairs as influenced by the various gas treatments.

GROWTH IN A GAS MIXTURE SIMILAR TO THAT OF NORMAL AIR

Two experiments were conducted to test the plant material and the experimental conditions. The roots of paired six-weeks-old plants in Woulff bottles were treated with a gas mixture similar to normal air in composition. In the first experiment 24 plants were used both in the aerated and in the unaerated series. A gas mixture consisting of 0.3 per cent carbon dioxide, 20.0 per cent oxygen and 79.7 per cent nitrogen was passed through each of the treated cultures daily. A quantity in excess of the pore space was removed from each culture. The plants were maintained at a temperature of $27 \pm 1°$ C under continuous illumination for three weeks. At the end of the experiment the growth of roots in the two cases showed no significant differences.

A second experiment, using two series of 30 plants each in culture tubes, was conducted in a fashion similar to the previous one. However, the daily renewals of gas were accomplished by withdrawing a much smaller quantity (usually 75 to 80 cc.) from each culture. The volume of gas removed was approximately three times the pore space occupied by the soil gas. A temperature of $25 \pm 1°$ C was maintained for the 15 days of the experiment.

A similar development was again noted with the plants of the treated and control series, all measured differences being well within

the limits of the probable errors. It should be stated that although the gas mixture used for the treatments was similar to air in composition, the gas withdrawn from the treated cultures contained less oxygen and more carbon dioxide than did the gas from the gasometer or from the open control cultures. For example, the gas analyzed from the treated tube cultures had an average composition of 2.1 per cent carbon dioxide, 16.6 per cent oxygen, and 81.1 per cent nitrogen as contrasted to that withdrawn from the control cultures which was 0.6 per cent carbon dioxide, 20.5 per cent oxygen, and 78.9 per cent nitrogen. This change in composition was undoubtedly associated with the respiratory activity of the plant roots which resulted in an accumulation of carbon dioxide and a disappearance of oxygen in the treated cultures. A similar condition was precluded in the open control cultures by the continuous gaseous interchange with the outside air.

GROWTH IN MIXTURES OF REDUCED OXYGEN AND INCREASED NITROGEN

Two experiments were conducted with Woulff bottles as follows: In the first experiment, a gas mixture with a very low oxygen content (1.2%) was administered to the roots of seven-weeks-old plants. These plants were grown for 17 days under the usual artificial illumination and at a temperature of 28 ± 1° C. Instead of the paraffin-petrolatum mixture, a soft modelling clay, 'plasteline,' was used for sealing in the plants. Analyses of the soil gas are reported in table 8 and the growth of roots in table 9.

TABLE 8

COMPOSITION OF THE SOIL GAS IN WOULFF-BOTTLE CULTURES WITH REDUCED OXYGEN SUPPLY

Culture	Day of experimental period	Number of analyses	Per cent CO_2	Per cent O_2	Per cent N_2
Gas-treated	3	6	1.2±0.11	*4.0±0.33	94.8±0.32
	6	6	1.0±0.03	1.5±0.29	97.5±0.30
	9	6	1.2±0.03	0.8±0.17	98.0±0.17
	13	6	0.9±0.02	0.6±0.09	98.5±0.09
	16	6	0.8±0.01	0.6±0.13	98.6±0.14
	Average		1.0±0.03	1.5±0.18	97.5±0.20
Control	16	6	0.6±0.08	20.4±0.09	79.0±0.03
Gas from tank		8	0.2±0.06	1.2±0.09	98.6±0.12

* The high oxygen content may have been due to some residual oxygen in the cultures during the first few days of the experimental period.

TABLE 9

Growth of Sour-orange Plants in Woulff-bottle Cultures with Low Oxygen Content*

Temperature 28 ± 1° C

Culture	Number of plants	Stem		Leaves		Tap root elongation	Lateral roots		Total root elongation
		Diameter	Height	Number	Fresh weight		Number	Elongation	
		cm.	*cm.*		*gm.*	*cm.*		*cm.*	*cm.*
Gas-treated	24	.14± 002	6.7±0 14	2 2±0.04	0.09± 005	0 0	0 0	0.0	0.0
Control	22	.14±.002	7.6±0 22	3 2±0.12	0.10±.006	2.3±0 35	5.9±0.63	6 4±0.87	8.7±0.94

* See table 8 for experimental conditions.

It will be seen from table 9 that the root elongation of the treated plants was entirely checked. Since the carbon dioxide percentages of the soil atmosphere of the treated and the control cultures were of the same magnitude, it seems highly probable that the suppression of root elongation with the treated plants was due to the deficiency of oxygen in the gas enveloping the plant roots.

In a second experiment with Woulff-bottle cultures, a gas mixture of a high oxygen content (8%) was administered to the roots of five-weeks-old plants with the temperature maintained at 30 ± 1° C. In order to diminish quickly the oxygen content of the treated cultures, a gas containing only 1 per cent of oxygen was used for the first two days, after which a second mixture containing about 8 per cent of oxygen was employed for 13 days more. This resulted in a low initial oxygen content of the treated jars (table 10), but subsequent analyses showed a relatively constant average oxygen content of about 7 per cent.

TABLE 10

Composition of the Soil Gas in Woulff-bottle Cultures with Reduced Oxygen Supply

Culture	Day of experimental period	Number of analyses	Per cent CO_2	Per cent O_2	Per cent N_2
Gas-treated	2	7	0 8±0 03	2 6±0 43	96 6±0 45
	5	7	1 1±0 02	6.6±0 04	92 3±0.05
	9	7	1 2±0 02	7 2±0.06	91 6±0 07
	12	7	1 3±0 03	6.9±0 05	91 8±0.05
	15	7	1.2±0 03	7 0±0 14	91 8±0.12
	Average		1 1±0 02	6 1±0 21	92 8±0.23
Control	15	7	0.6±0 07	20 4±0 05	79.0±0.03
Gas from tank		6	0.5±0 08	7.9±0.52	91 6±0.50

TABLE 11

GROWTH OF SOUR-ORANGE PLANTS IN WOULFF-BOTTLE CULTURES WITH LOW OXYGEN CONTENT*

Temperature 30 ± 1° C

Culture	Number of plants	Stem		Leaves		Tap root elongation	Lateral roots		Total root elongation
		Diameter	Height	Number	Fresh weight		Number	Elongation	
		cm.	*cm.*		*gm.*	*cm.*		*cm.*	*cm.*
Gas-treated	28	.17±.002	7.6±0.07	2.6±0.14	.09±.003	0.8±0.17	3.5±0.55	2.0±0.48	3.8±0.57
Control	28	.17±.002	7.9±0.09	3.4±0.11	.11±.003	2.4±0.36	7.4±0.40	7.4±0.42	9.8±0.63

* See table 10 for experimental conditions.

The effect of this treatment upon plant growth (table 11) may be summarized briefly as follows: When the oxygen content of the soil atmosphere was reduced to between 6 and 7 per cent, the root growth of the plants was only about one-third to one-half that of the control plants which had a soil atmosphere of nearly the same composition as normal air.

Two other experiments, both with culture tubes, were conducted with a gas mixture of low oxygen content. In the first experiment six-weeks-old plants were used and the tubes sealed with a paraffin-petrolatum mixture of a high melting point (about 52° C). It was found necessary to reseal the treated cultures twice during the experiment in order to stop leaks around the plants. This was done with the aid of a hot needle; unfortunately some of the plants were injured and had to be discarded. Much less resealing was done with the control cultures. The gas in the treated tubes was changed twice daily by drawing through each tube a quantity (85 cc.) equal to about three times the pore space occupied by the soil gases. Growth was allowed to take place for 15 days at a temperature of 25 ± 1° C. Analyses of the soil gas are given in table 12.

TABLE 12

COMPOSITION OF THE SOIL GAS IN TUBE CULTURES WITH REDUCED OXYGEN SUPPLY

Culture	Day of experimental period	Number of analyses	Per cent CO_2	Per cent O_2*	Per cent N_2
Gas-treated	4	13	0.9±0.05	5.1±0.31	94.0±0.28
	9	13	1.2±0.08	3.8±0.22	95.0±0.18
	14	13	1.6±0.09	5.0±0.58	93.4±0.54
	Average		1.2±0.06	4.6±0.21	94.2±0.19
Control	15	15	0.6±0.05	20.4±0.06	79.0±0.03
Gas from tank		5	0.2±0.02	3.0±0.03	96.8±0.05

* The high oxygen content of the treated cultures, as compared with the tank, is due to the presence of air which was drawn in through leaks in the system during the daily gas changes.

TABLE 13

Growth of Sour-orange Plants in Tube Cultures with Low Oxygen Content*

Temperature 25 ± 1° C

Culture	Number of plants	Stem		Leaves		Tap root elongation	Lateral roots		Total root elongation
		Diameter	Height	Number	Fresh weight		Number	Elongation	
		cm.	*cm.*		*gm.*	*cm.*		*cm.*	*cm.*
Gas-treated	20	.17±.002	8.0±0.11	2.2±0.04	.12±.003	0.8±0.14	6.1±1.03	4.6±0.89	5.4±0.92
Control	27	.16±.002	8.2±0.10	2.6±0.13	.13±.003	3.3±0.25	8.6±0.55	9.3±0.80	12.6±0.91

* See table 12 for experimental conditions.

The effects of this gas mixture upon plant growth are shown in table 13. The data indicate that the total root elongation of the treated plants was only about one-half that of the control plants when the soil atmosphere consisted of 4 to 5 per cent oxygen and the remainder almost exclusively nitrogen.

A second experiment was conducted with tube cultures using a somewhat higher oxygen content (about 8%). A lower-melting wax mixture (m.p. 42° C) was used with the result that better seals were secured, but a few plants were injured, apparently by paraffin infiltration. The gas in the treated cultures was changed twice daily by drawing about 125 cubic centimeters through each tube. A temperature of 25 ± 1° C was maintained during the experimental period of 12 days.

A considerable increase of carbon dioxide in the treated cultures (table 14) was found during the experiment. It is thought that this increase was due not only to the respiratory activity of the roots but also to some residual carbon dioxide in the water in the gas tank, which gradually escaped from solution and increased the carbon dioxide content of the gas mixture.

TABLE 14

Composition of the Soil Gas in Tube Cultures with Diminished Oxygen Supply

Culture	Day of experimental period	Number of analyses	Per cent CO_2*	Per cent O_2	Per cent N_2
Gas-treated	3	15	1.2±0.08	8.7±0.18	90.1±0.17
	8	15	2.3±0.10	7.7±0.25	90.0±0.21
	12	15	4.8±0.14	7.7±0.38	87.5±0.19
	Average		2.8±0.19	8.0±0.17	89.2±0.18
Control	11	15	0.6±0.05	20.3±0.04	79.1±0.01
Gas from tank		7	2.5±0.40	8.6±0.10	88.9±0.35

* See text for explanation of the increasing carbon dioxide percentages.

TABLE 15

Growth of Sour-orange Plants in Tube Cultures with Low Oxygen Content*
Temperature 25 ± 1° C

Culture	Number of plants	Stem		Leaves		Tap root elongation	Lateral roots		Total root elongation
		Diameter	Height	Number	Fresh weight		Number	Elongation	
		cm.	*cm.*		*gm.*	*cm.*		*cm.*	*cm.*
Gas-treated	26	.17±.002	7.5±0.11	2.1±0 01	.11±.002	0.8±0 14	5 0±0 63	2 1±0.32	2.9±0 44
Control	26	.16±.002	7.6±0.09	2.3±0.05	.11±.003	1.8±0.24	7.5±0.59	4 5±0 44	6.3±0.61

* See table 14 for experimental conditions.

A considerable retardation of root growth was again evident, even with an average oxygen content of 8 per cent (table 15). It was surprising to find that the hydrogen-ion concentration of the extracted solution was very low, pH 8.5 for the treated cultures and pH 9.2 for the control cultures. It was pointed out to the writer that the nature of the minerals found in the soils of the region from which the sand was obtained is such as to give the soil solution an alkaline reaction. This may account for the high pH values obtained for the solutions withdrawn from the sand cultures. Apparently the greater carbon dioxide content of the treated cultures was responsible for their more acid reaction.

GROWTH IN HIGH CONCENTRATIONS OF CARBON DIOXIDE

It was desired to determine whether carbon dioxide exerts a retarding influence upon the growth of Citrus plants. In order that oxygen might not be a limiting factor, it appeared desirable to supply the same quantity of oxygen to the treated cultures as the controls would receive from the surrounding air. Six-weeks-old sour-orange seedlings were grown in culture tubes with a soil atmosphere of high carbon dioxide (55%) and normal oxygen content for 15 days. The temperature during this period was maintained at 25 ± 1° C. A wax with a rather high melting point was used for sealing in the plants and it again proved necessary to resort to the resealing of leaky cultures. This resulted in injury to some of the plants, particularly with the treated cultures which were resealed more often, so that many of the plants had to be discarded.

A considerably lower carbon dioxide content was found in the treated cultures than existed in the tank (table 16). This and the high probable errors of the tank analyses, were evidently the result

of dilution by the outside air which was drawn into the system through occasional leaks. The rather large probable errors in the tank analyses were due to a fluctuating composition associated with occasional additions of quantities of gas.

TABLE 16

COMPOSITION OF SOIL GAS IN THE TUBE CULTURES WITH INCREASED CARBON DIOXIDE SUPPLY

Culture	Day of experimental period	Number of analyses	Per cent CO_2*	Per cent O_2	Per cent N_2
Gas-treated	5	13	53.3±2 03	20.4±0.16	26.3±2.08
	10	13	54 6±2 80	19.9±0.18	25 5±2 89
	14	13	55 7±2 37	18.3±0.38	26 0±2.11
	Average		54 5±1 34	19.5±0 17	26.0±1.32
Control	13	14	0 4±0.03	20.5±0 03	79 1±0.01
Gas from tank		12	74 2±0.41	20.7±0.43	5.1±0.35

* See text for explanation of the low carbon dioxide content of the treated cultures as compared to the tank.

TABLE 17

GROWTH OF SOUR-ORANGE PLANTS IN TUBE CULTURES WITH HIGH CARBON DIOXIDE CONTENT*

Temperature 25 ± 1° C

Culture	Number of plants	Stem		Leaves		Tap root elongation	Lateral roots		Total root elongation
		Diameter	Height	Number	Fresh weight		Number	Elongation	
		cm.	*cm.*		*gm.*	*cm.*		*cm.*	*cm.*
Gas-treated	19	.13± 002	9.3±0 16	2 0±0.00	.09± 004	0 08±0 02	0.1±0 04	0 01± 004	0 09±0 02
Control	25	15± 002	9 7±0 12	2 8±0.12	.12±.005	3 4±0 35	9.3±0 46	7.7±0 63	11 1±0 75

* See table 16 for experimental conditions.

The data presented in table 17 indicate that growth was practically suppressed by the large percentage of carbon dioxide in the soil atmosphere of the treated cultures. It should be remembered, however, that the effects produced by the necessary resealing may be a contributing factor in the case of the treated plants retained.

In a second experiment of this type a wax with a lower melting point (m.p. about 35° C) was used for sealing around the plants. Although the resulting seals were fairly effective, the mixture showed evidence of infiltrating into some of the plant stems so that the experiment had to be terminated on the eighth day. The experimental conditions were in general the same as those for the previous experiment,

including semi-daily gas renewals for the treated cultures, except that a lower carbon dioxide content was employed. The data for this experiment are briefly summarized in table 18.

TABLE 18

GROWTH OF SOUR-ORANGE PLANTS IN TUBE CULTURES WITH A HIGH CARBON DIOXIDE CONTENT

Temperature 25 ± 1° C

Culture	Gas content			Number of plants	Tap root elongation	Lateral roots		Total root elongation
	Per cent CO_2	Per cent O_2	Per cent N_2			Number	Elongation	
					cm.		*cm.*	*cm.*
Gas-treated	37.4±1.06	16.5±0.15	46.1±1.04	20	0.0	0.0	0.0	0.0
Control	0.4±0.02	20.5±0.02	79.1±0.02	27	0.6±0.11	2.6±0.59	0.6±0.13	1.2±0.23

In general it may be concluded that the presence of high concentrations of carbon dioxide in the soil atmosphere resulted in a definite retarding effect upon the root elongation of sour-orange seedlings. The fact that some of the treated plants died during the course of the experiment, while those discarded from the controls were only injured, suggests that the carbon dioxide was an important factor in the death of some of the treated plants.

DISCUSSION

The results of the soil-gas studies presented in this section may be briefly summarized in the following statements: Check experiments conducted with gaseous mixtures similar to that of the air showed that normal growth (as compared with control plants) may be secured under the conditions of the experiment. Treatments, in which soil gases of diminished oxygen content were employed, resulted in a considerable reduction of root growth. For example, the root elongation in gaseous mixtures containing 5 or 8 per cent oxygen and the remainder chiefly nitrogen was only about one-half that of the control plants at a temperature of 25° C. A further reduction of the oxygen content to a value of 1.2 to 1.5 per cent resulted in the complete suppression of root growth at 28° C. Finally, the presence of a high carbon dioxide content (37 to 55%) in the soil gas was found to result in an almost complete suppression of root growth at a temperature of 25° C, even though the oxygen content approached that of normal air. Not only was root growth inhibited but some plants showed definite injury which was apparently due to the carbon dioxide treatment.

The question arose whether the treatment with carbon dioxide gave the solution an acid reaction which in turn resulted in suppressing root growth. In order to test this possibility ten culture tubes were set up as described for the soil-gas studies, but without plants. After semi-daily gas treatments for a period of four days with a gas mixture containing a high carbon dioxide concentration, the gas contents of the tubes were analyzed and the pH values of the extracted solutions determined. The average gas composition for the ten tubes was 70.2 per cent carbon dioxide, 5.5 per cent oxygen, and 24.3 per cent nitrogen. The pH determinations showed an average value of 7.56 ± 0.02. It is therefore evident that the reaction of the soil solution was not the factor limiting root growth, since the hydrogen-ion concentration of the gas-treated cultures was nearer the optimum for growth than that of cultures having only a small amount of carbon dioxide and previously found to be decidedly alkaline (8.5 to 9.2).

The results obtained in these studies agree, in general, with those obtained by different investigators for various plants. Cannon (1925) has worked extensively in this field and has studied the soil-gas relations of a large number of plants. His findings indicate that, while considerable difference of behavior existed with various species, practically all required an appreciable amount of oxygen present in the soil for continued root growth. Furthermore, it was found that the oxygen requirement for root growth increased with temperature, i.e., an oxygen content which permitted normal root growth at a given temperature might act to limit growth at a higher temperature. In the same way it was observed that different plants reacted somewhat differently toward large concentrations of carbon dioxide in the soil atmosphere, and that such concentrations might prove toxic for many plants. For example, the growth of *Opuntia* was entirely checked by exposure to a mixture of 25 per cent oxygen and 75 per cent carbon dioxide for a short period, but growth was quickly resumed with the admission of atmospheric air. Varying concentrations of carbon dioxide diluted with air or oxygen were found to depress root growth in *Covillea, Prosopis, Opuntia,* and *Krameria.*

In addition, Cannon has recorded the results of some experiments with Citrus plants. He reports that sweet-orange plants were able to withstand high concentrations of carbon dioxide (75% CO_2 and 25% O_2 at 20 to 25° C) for a period of four days, but that the resulting growth was slow. Cannon states that, in the absence of carbon dioxide, sweet-orange and rough-lemon plants gave evidence of con-

tinued root growth with 2.5 per cent oxygen, but that root growth was inhibited when the oxygen content was reduced to 2.0 per cent. The minimum oxygen content for root growth with sour-orange plants was thought to be somewhat lower than that for the sweet orange. These values were obtained for short experimental periods (1 to 5 days) and represent measurements made with a comparatively small number of plants giving results of a doubtful statistical reliability.

Noyes, Trost, and Yoder (1918) have also studied the effects of high concentrations of carbon dioxide upon the growth of various plants. The continuous passage of carbon dioxide through the soil in which Christmas pepper (*Capsicum annum abbreviatum*) plants were growing resulted in a stunting and dwarfing of the main roots. An intermittent treatment produced coarser and more 'clumped' roots than those of the control. Other plants studied showed somewhat similar effects but to a slighter degree.

The effects of these treatments upon the growth of plant roots must evidently depend upon a fundamental process (or processes) going on within the plant tissues. It has been suggested by Livingston and Free (1917) that the process of respiration is the one primarily concerned in this connection. The effect of a low content of available oxygen would therefore be to retard aerobic respiration by causing oxygen to become a limiting factor.

GENERAL DISCUSSION

It seems evident from the experimental results obtained that the root growth of Citrus seedlings may be markedly influenced by changes in certain of the environmental factors. Extreme changes in any one factor may result in profound changes in plant growth.

Moderate changes in one or more of the environmental factors, however, may or may not produce significant changes in plant growth. When these variations occur well within the range favorable to plant growth, the response of the plant is likely to be small. When, on the other hand, the changes are near either extreme of the range, the response appears to be greater. Thus temperatures of 27° and 31° C were associated with values of 20.5 and 19.9 centimeters respectively, for the average root elongation obtained with populations of 30 sour-orange plants. But an increase from 13° to 18° C was accompanied by an increased root elongation of 13.5 centimeters, and an increase from 34° to 37° C resulted in a reduction of root elongation from 10.2 to 1.4 centimeters.

It is of interest to apply the findings of this investigation to an interpretation of growth under field conditions. For example, unpublished data collected by the Division of Orchard Management of the Citrus Experiment Station give 46° F (approximately 8° C) as the minimum soil temperature and 89° F (approximately 32° C) as the maximum for 1925. These temperatures were recorded for a soil depth of one foot at Riverside, California. It is evident that they represent a range including both subminimal and superoptimal temperatures for root elongation.

A second important factor is the reaction of the soil solution. Usually the reaction of the soils in the citrus regions of southern California is definitely alkaline. In certain cases soil alkalinity may be the factor limiting growth, and perhaps, to a still greater degree, limiting root-hair production.

In view of the responses to increased aeration obtained with sour-orange roots, it seems evident that the aeration of the soil must be an important factor influencing root growth under field conditions. Thus, such conditions as a 'tight' soil, or the occurrence of a plow-sole, irrigation hardpan, or other compacted soil layers which act to restrict soil aeration, may undoubtedly exert a profound influence upon root growth. The application of excessive amounts of water to the soil also acts to restrict aeration. The respiratory activity of the roots under such conditions results in a soil air poor in oxygen and rich in carbon dioxide. Both of these conditions may operate to limit the growth of sour-orange roots. On the other hand, the situation occurring in well aerated, well drained soils undoubtedly leads to maximum root growth, other conditions being favorable.

In general it is evident that root growth in the field is conditioned by various factors which may be closely interrelated and may often operate in a very complex manner. The experimental results herein described, and their application to field conditions, must therefore be qualified by making allowance for the special experimental conditions and for the more complex situation associated with the growth of plants in the field.

SUMMARY

1. The root growth of seedlings of grapefruit, sour-orange, and sweet-orange in solution cultures was found to have a minimum temperature of approximately 12° C, an optimum temperature of 26° C, and a maximum temperature of approximately 37° C.

2. The optimum temperature for the production of root hairs for sweet- and sour-orange plants was apparently somewhat higher than that most favorable to root elongation—approximately 33° C as contrasted with 26° C.

3. The root growth of sour-orange seedlings in solution cultures gave evidence of being significantly influenced by the reaction of the solution. When the hydrogen-ion concentrations were adjusted twice daily it was found that a minimum of root elongation occurred at or below pH 4.0, a maximum at pH 6.5 (or approximate neutrality), and a second minimum at or above pH 9.0.

4. The hydrogen-ion concentration most favorable for the production of root hairs was found to be distinctly acid, pH 5.0.

5. A continuous aeration of the culture solution was found to give increased root growth as measured by the elongation of the tap and lateral roots.

6. The production of root hairs was greatly enhanced by aeration. The data indicate that root-hair formation was much more responsive to the aeration treatment than was root elongation.

7. Data secured from sour-orange seedlings grown in sand cultures indicate that root elongation was entirely suppressed by a very low oxygen content of the soil atmosphere (1.2 to 1.5% at 28° C), and that a retarding influence was evident when the oxygen content was considerably higher (5 to 8%).

8. The root growth of sour-orange plants in sand cultures was also found to be adversely affected by high concentrations of carbon dioxide in the soil atmosphere. Total root elongation was suppressed with 37 to 55 per cent carbon dioxide at a temperature of 25° C, even though the oxygen content (17 to 20%) of the soil atmosphere was not greatly below that of normal air.

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